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The bacterial and fungal community assemblages in the Eucalyptus phyllosphere are shaped by contrasting ecological processes.

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Research Article

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Abstract

Plant performance and ecosystem functioning are inextricably tied to the phyllosphere microbiome. However, little is known about how ecological processes influence the biogeography of various phyllosphere microbial populations. Here, we examined the biogeographic patterns of fungal and bacterial communities in phyllosphere samples of plants from the Myrtaceae family's species Eucalyptus that were taken across a distance of more than 4000 km. We examined how the two main ecosystem processes—stochastic and deterministic—affect the assembly of microbial communities in the phyllosphere. Our findings showed that 64.2% of the fluctuations in bacterial community assembly, which had a normalised stochasticity ratio of 71.8%, were described by the neutral community model, which can measure the degree of a community assembly caused by stochastic processes. These findings imply that stochastic processes play a major role in influencing the formation of bacterial communities. On the other hand, a normalised stochasticity ratio of 25.1% and a comparatively low explanation rate of the neutral community model (48.7%) indicate that deterministic mechanisms dominated the formation of phyllosphere fungal communities. The findings of random forest modelling and variation partitioning analysis showed that both bacterial and fungal bio-geographic patterns in the phyllosphere were driven by climatic parameters, especially mean annual precipitation and aridity index. Fungal community assembly was more strongly correlated with edaphic variables than bacterial community assembly, such as soil organic and mineral nitrogen concentration. The distinct characteristics of bacteria and fungus, such as the greater potential dispersion rate and broader habitat niche of bacteria compared to fungi, may be responsible for the varied reactions of bacterial and fungal populations to environmental conditions. The "size plasticity" theory, which holds that smaller organisms (bacteria) are more homogeneous across space and less affected by environmental conditions than larger organisms (fungi), is supported by our findings, which showed that phyllosphere bacterial and fungal communities followed distinct community assembly processes. These results provide fresh perspectives on the microbial ecology of microbiomes in the phyllosphere of plants.

KEYWORDS

community formation, stochastic processes, phyllosphere microbiome, plant microbiome, and deterministic processes

1 | INTRODUCTION

One of the main topics in microbial ecology is comprehending the ecological mechanisms that control the processes of microbial community formation.^{1,2} According to neutral-based theory, stochastic processes play a major role in community assembly and all members of a community are ecologically equal with the same demographic rates.³ In contrast, niche-based theory holds that the assembly of microbial communities is determined by deterministic processes mediated by variations in biotic (such as competition, facilitation, and predation among microorganisms) and abiotic (such as pH and temperature) environmental conditions.^{4,5} After much discussion, it is now generally acknowledged that, rather than being mutually exclusive, stochastic and deterministic mechanisms work together to regulate microbial community assemblages in natural ecosystems.⁶⁻⁸ Understanding microbially driven ecosystem processes and functions requires quantifying the relative contributions of stochastic and deterministic processes to microbial community assemblies, since responses of microbial genes to environmental and ecological influences vary across communities and habitats.⁷⁻⁹ There is now growing evidence that the assembly patterns of bacterial and fungal communities differ. For instance, Bahram et al. discovered that the fungal community exhibited a more noticeable diversity pattern than the bacterial community in terrestrial habitats.¹⁰ Chen et al.⁷ found that deterministic mechanisms had a stronger effect on the fungal community than the bacterial community in termite mounds. These findings could be explained by variations in the traits of fungus and bacteria. The majority of fungus often have bigger bodies than bacterial cells and multicellular hyphal structures, in contrast to unicellular bacteria. Furthermore, fungus and bacteria often have distinct preferences for habitats.^{11, 12} These distinctions between fungal and bacterial cells may have an impact on their dispersal capacity, metabolic processes, and reactions to environmental filters.^{12, 13} The significance of these processes in identifying the community assemblages in the plant phyllosphere—the aerial portions of plants—remains largely unanswered despite the increased interest in this topic. The microbiome of the phyllosphere has significant agronomic and ecological significance. Microbes that live in the phyllosphere may affect host plants in both positive and negative ways.¹⁴⁻¹⁶ Certain beneficial phyllosphere microorganisms, for instance, can support plant growth by investigating how various ecological mechanisms influence the assemblies of bacterial and fungal communities, the relative supply of nutrients and cofactors (such as non-protein compounds that act as catalysts of enzyme activity), the enhancement of plant hormone production, and the increase of plant tolerance to stresses (such as drought) by contributing to the fitness of plants.^{14, 17-19} Pathogenic phyllosphere microorganisms, on the other hand, may endanger plant health and negatively affect plant development and performance.^{20,21} By aiding in the fixation of nitrogen and the breakdown of organic materials, phyllosphere microbes also contribute significantly to global biogeochemical cycles.²² Our ability to comprehend the ecological roles of phyllosphere microbiota and to use phyllosphere microbial communities for controlling plant disease and boosting crop productivity is severely hampered by the knowledge gap regarding how various ecological processes shape phyllosphere microbial community assemblies. In this work, we investigated the phyllosphere microbiome of plants in eastern and northern Australia that belong to the *Eucalyptus* genus of the Myrtaceae family. We examined the biogeographic trends of fungal and bacterial populations in the phyllosphere. We postulated that distance-decay relationships (DDRs) were present in the similarity of both phyllosphere bacterial and fungus populations. Since bacteria can adapt to a wider variety of environmental circumstances than fungus, we also hypothesised that the bacterial

community is more controlled by stochastic processes and is less significantly impacted by environmental filtration than the fungal population.^{23, 24} We used the neutral community model (NCM)²⁵ to verify these theories, and we computed the normalised stochasticity ratio (NST) of fungus and bacteria to quantitatively evaluate ecological stochasticity in phyllosphere microbial community assemblies. Furthermore, we computed habitat niche breadth (Bcom), another crucial characteristic that affects how deterministic and stochastic processes contribute to the formation of microbial communities. Using Spearman's correlation analysis, variation partitioning analysis (VPA), and random forest modelling, we further investigated the connections between the composition of phyllosphere bacterial and fungal communities and specific climatic and edaphic parameters. The goal of this work was to offer a thorough knowledge of the mechanisms involved in the formation of microbial communities in the phyllosphere of plants and to determine how various microbial groups are affected differently by environmental filtration.

2 | MATERIALS AND METHODS

2.1 | Sampling campaign

In May 2019, samples of plant leaves and soil were taken from 112 sites in eastern and northern Australia (133.42° E to 153.60° E, -38.19° S to -19.16° S; Figure 1). A handheld GPS device (eTrex Venture; Garmin) was used to capture the spatial geographical coordinates of the sample sites. Every sample site has a 50 x 50 m sampling

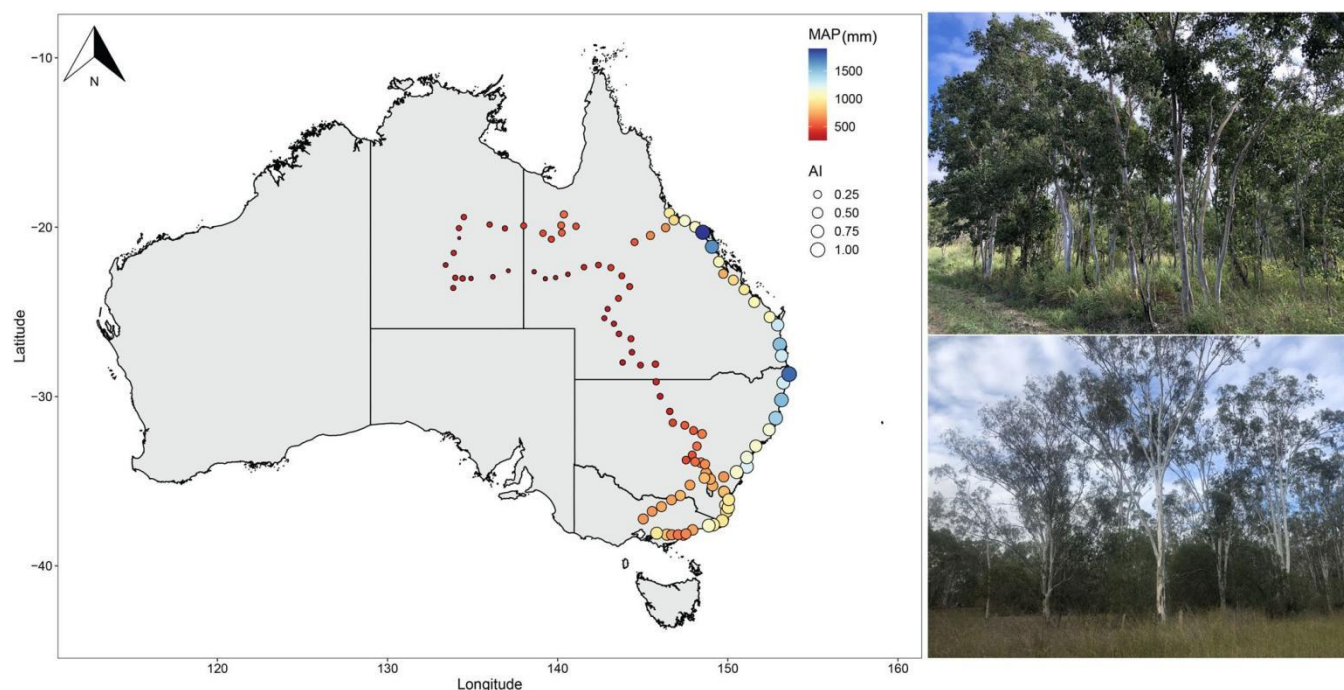


FIGURE 1: Images of eucalyptus plants and sampling locations in northern and eastern Australia. The figure was created using the colour and size of the blotches according to the aridity index (AI) and mean annual precipitation (MAP), respectively. Using sterile scissors, leaf samples from five distinct Eucalyptus trees were taken in each plot to create a composite sample. Five soil cores from the top 10 cm were mixed to create a reference soil sample for every composite leaf sample. This sampling session resulted in the collection of 112 soil samples and 112 leaf samples. The gathered samples were sent right away to the lab for examination.

2.2 | Climate data collection

Based on the sites' geographic coordinates, the WorldClim database version 2 (<http://worldclim.org/>) provided the mean annual temperature (MAT) and mean annual precipitation (MAP) for each sample location.²⁶ The sample region's MAT and MAP varied between 254 and 1901 mm and 12.93 and 26.35° C, respectively. Using information gathered from the Climate Database V2 (<https://cgiarcsi.community/>), the aridity index (AI), which is the ratio of precipitation to evapotranspiration, was computed. The sample region's AI varied between 0.08 and 1.08. Table S2 contains comprehensive data for each location's climatic variables.

2.3 | Soil physicochemical characterization

Standard techniques were used to characterise the soil samples' physicochemical characteristics.²⁷ In short, a pH meter (Thermo Fisher Scientific Inc.) was used to test the pH of soil in a 1:2.5 soil:water solution. A total organic carbon analyser (Shimadzu) was used to quantify the dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) that were extracted using MilliQ® water (Merk & Co.,³ Inc.). Mineral nitrogen, such as nitrate-nitrogen (NO⁻-N) and ammonium nitrogen (NH⁺-N), was extracted using a 2 M KCl solution and measured using a flow analyser (Skalar Analytical B.V. Tinststraat). Table S1 lists each location's soil characteristics.

2.4 | Microbial community characterization

The extraction of genomic DNA from the plant phyllosphere was carried out using an earlier technique.²⁸ Using sterile scissors, the plant leaf samples were chopped into tiny pieces. A 25 ml sterile flask with 100 ml of 0.01 M autoclaved phosphate-buffered saline (PBS) was filled with five grams of leaf tissue. Using a shaking incubator, the leaf tissue and PBS combination was sonicated for seven minutes and then shaken for one hour at 180 rpm and 30° C. Following shaking, a 0.02 µm cellulose membrane and nylon gauze were used to filter the mixture. Following the manufacturer's instructions, the cellulose membrane was sliced into tiny pieces and used for DNA extraction using a DNeasy PowerSoil Kit (QIAGEN Pty, Ltd.). A Qubit™ dsDNA HS Assay kit on a Qubit™3.0 fluorometer (Thermo Fisher Scientific Inc.) was used to quantify the DNA concentration. A CFX96 Touch™ PCR detection system (Bio-Rad) was used to measure the copy numbers of the fungal ITS region and the bacterial 16S rRNA gene. The primer pair 515FmodF/806RmodR.²⁹ was used to amplify the bacterial 16S rRNA gene. The primer pair ITS1F/ITS1R was used to amplify the fungal ITS region. At Majorbio Bio-Pharm Technology Co., Ltd., 30 amplicons were sequenced using an Illumina MiSeq PE300 platform (Illumina Inc.). MajorBio filtered raw sequences for quality assurance. In order to produce high-quality sequences, raw pair-ended reads with an average quality score <20, more than three ambiguous nucleotides, or a length <100 nt were eliminated along with barcodes. The Quantitative Insight Into Microbial Ecology (QIIME) was used to analyse and interpret the high-quality sequences that were acquired.³¹ Singletons, mitochondrial, chloroplast, and chimeric sequences were found using QIIME and removed from the final data set. The SILVA data collection for bacteria³² and the UNITE data set for fungi were used to assign representative sequences to operational taxonomic units (OTUs).³³

2.5 | Statistical analyses

At the $p < 0.05$ level, statistical findings were deemed significant. The "ggplot2" package³⁴ in R.³⁵ was used to visualise the diversity and abundance of bacteria and fungus. Using the "stat" package and the Wilcoxon rank-sum test, the variations in bacterial and fungal abundances were ascertained.³⁶ By computing each pairwise Spearman's correlation coefficient, co-occurrence networks of bacterial and fungal species at the OTU level were created. If Spearman's correlation coefficient (ρ) > 0.8 and $p < 0.01$.³⁷, two taxa were deemed correlated. Co-occurrence networks built using Gephi 0.9 were used to visualise the resultant correlation matrix. Two. The bacterial and fungal communities' DDRs were determined by calculating the slope of the ordinary least square (OLS) regression between community

similarity and geographic distance (1 - [Bray-Curtis dissimilarity value]). Nonmetric multidimensional coordination (NMDS) based on Bray-Curtis distance computed using the "vegan" package was used to visualise the community profiles of bacterial and fungal communities.³⁸ Analysis of similarities (ANOSIM) was used to determine the significance of differences between several groups of microorganisms [classified based on the climate of the sample locations]. According to Sloan et al.²⁵, the NCM was used to forecast the possible significance of stochastic events in impacting microbial community assembly using R code supplied by Burns et al.³⁹. To further measure the significance of stochastic and deterministic processes in determining microbial community assembly, the NST values for bacterial and fungal communities were computed. Forty The bacterial and fungal NST values were computed using the OTU tables derived from the sequencing of the bacterial 16S rRNA gene and the fungal ITS region, respectively. The "niche" was used to determine Levin's niche breadth values (Bcom) for bacterial and fungal populations. width" function under the package "spaa."⁴¹ Spearman's correlation analysis was used to examine the relationships between certain climatic and edaphic parameters and microbial abundance (16S rRNA gene or ITS region copies per gram sample), α -diversity (observed species), and community composition (NMDS1 value). VPA was carried out using "vegan" and the "varpart" function.³⁸ The "randomForest" software was used to create random forest modelling.⁴² The "rfPermute"⁴³ and "A3" packages were used to assess the importance of the rise in MSE (%) for each predictor.

RESULTS

2.6 | Characteristics of bacterial and fungal communities

Bacteria were substantially ($p < 0.001$) less common than fungi in the phyllosphere samples (Figure 2a). The samples' bacterial abundance (16S rRNA gene copies/g sample) varied from 5.59×10^4 to 1.80×10^9 , with an average of 6.79×10^7 . The average number of fungus (ITS copies/g sample) was 4.52×10^8 , with a range of 4.25×10^4 to 1.07×10^{10} . The average α -diversity (observed species) of the bacterial community in the samples was 587.33, with a range of 224 to 1224 (Figure S1). The average α -diversity of the fungal community in the samples was 691.83, with a range of 190 to 1659 (Figure S1). At a 97% similarity level, high-quality sequences were grouped into 8887 bacterial and 9864 fungal OTUs. The majority of samples shared a few abundant OTUs for both bacterial and fungal populations (Figure S2). In this case, dominating OTUs were those that were shared by more than 90% of samples (i.e., found in more than 100 samples). Twenty-three dominant OTUs were found for the bacterial community, accounting for 57.2% of all 16S rRNA gene reads. 29 prominent OTUs were found for the fungal community, accounting for 46.5% of all ITS readings. The sequences' higher-level taxonomic classification revealed the existence of 11 fungal and 44 bacterial phyla. The phylum Proteobacteria, which on average made up 77.8% of the overall bacterial population, comprised the majority of the bacterial community (Figure 2b). Ascomycota was the most prevalent phylum in the fungal community, accounting for 96.4% of the total fungal abundance on average (Figure 2b). Based on strong (Spearman's correlation coefficient $\rho > 0.8$) and significant ($p < 0.01$) correlations at the OTU level, network analysis was used to uncover the co-occurrence patterns of bacterial and fungal taxa and interkingdom interactions. With 61 correlations between bacterial taxa, 573 between fungal taxa, and 108 between bacterial and fungal taxa, the network consisted of 742 correlations (edges) among 197 taxa (nodes)

(Figure 2c). The network's average degree of connectedness was 7. The network's modularity score was 0.48, meaning that nodes inside modules had dense connections whereas nodes in other modules had sparse connections (Figures 2c and S4). Fungal and bacterial taxa have very distinct network topologies, with bacterial taxa having much reduced network connectedness (Figure 2c). Network hubs were identified as nodes with high degree (>30) and proximity centrality (>0.5) values. 33 network hubs that were all part of the same module were found (Figures S3 and S4; Table S1). The 33 network hubs were part of two

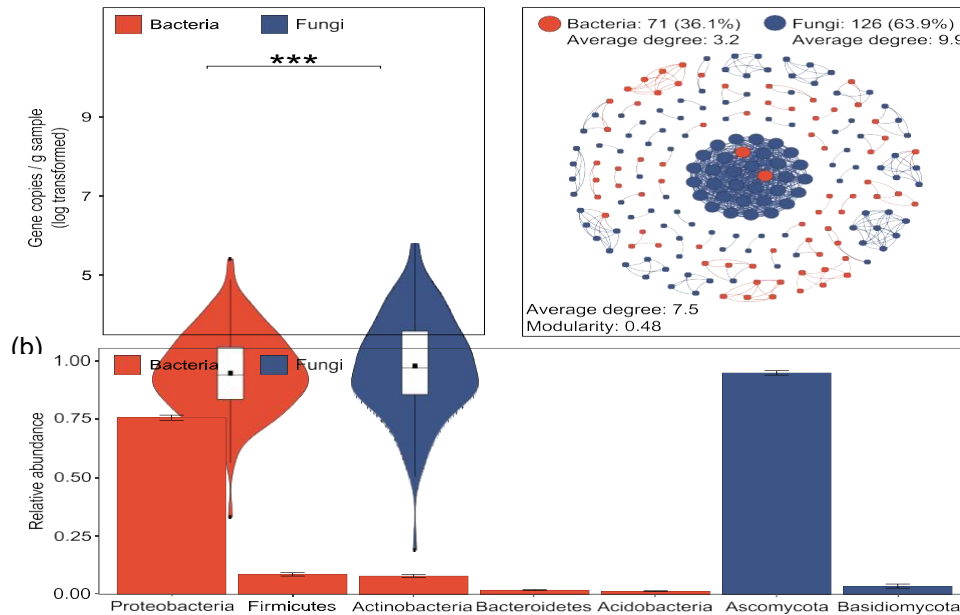


FIGURE 2: characteristic of the fungus and bacterial populations in the phyllosphere. (a) Bacterial and fungal community abundances are shown in box and violine plots. Significant Wilcoxon rank-sum test findings are shown by asterisks ($***p < 0.001$). (b) Bar plots displaying the top bacterial and fungal phylums' relative abundances. (c) Co-occurrence network of microbial taxa with strong ($\rho > 0.8$) and significant ($p < 0.01$) relationships at the species level. The number of correlations between two fungal taxa, Ascomycota and Basidiomycota, and bacterial phyla, Actinobacteria and Bacteroidetes, is shown by the size of the nodes (circle) (Figure S3, Table S1).

2.7 | Biogeographic patterns of bacterial and fungal communities

We calculated DDRs of the community similarities in order to examine the biogeographic trends of phyllosphere bacterial and fungal communities. Both communities showed a substantial distance decay trend, as seen by the significant ($p < 0.001$) negative correlations between geographic distance and community similarity (Figure 3a). The DDR curve for the fungus community was steeper (slope = -0.402) than that of the bacterial community (slope = -0.065), indicating that the fungal population may be more sensitive to environmental changes as geographical distance increases. The community compositions of phyllosphere communities altered significantly (ANOSIM $p = 0.001$) throughout the gradient of climatic parameters, including MAP, MAT, and AI, according to NMDS analysis (Figures 3b and S5). Microbial community assembly processes in plant phyllosphere We fitted bacterial and fungal OTUs to the NCM to estimate the occurrence of a given OTU and its relative abundance in order to investigate the relative significance of neutral and non-neutral processes in forming phyllosphere microbial community assemblies. 48.7% of the differences in the fungal community and 64.2% of the variations in the bacterial community were explained by the NCM (Figure 4a). Stochastic processes contributed more to the assembly of bacterial communities than to fungal communities, as seen by the greater explanation rate of NCM for bacterial communities compared to fungal communities. We calculated NST for bacterial and fungal communities in order to better define the relative importance of stochastic and

deterministic events in the phyllosphere microbial community assembly processes. The mathematical framework known as NST uses 50% as the border between more deterministic (<50%) and more stochastic (>50%). 40 NST for fungal and bacterial communities was 25.1% and 71.8%, respectively (Figure 4b). These findings showed that stochastic processes mostly formed the bacterial ecology in the phyllosphere.

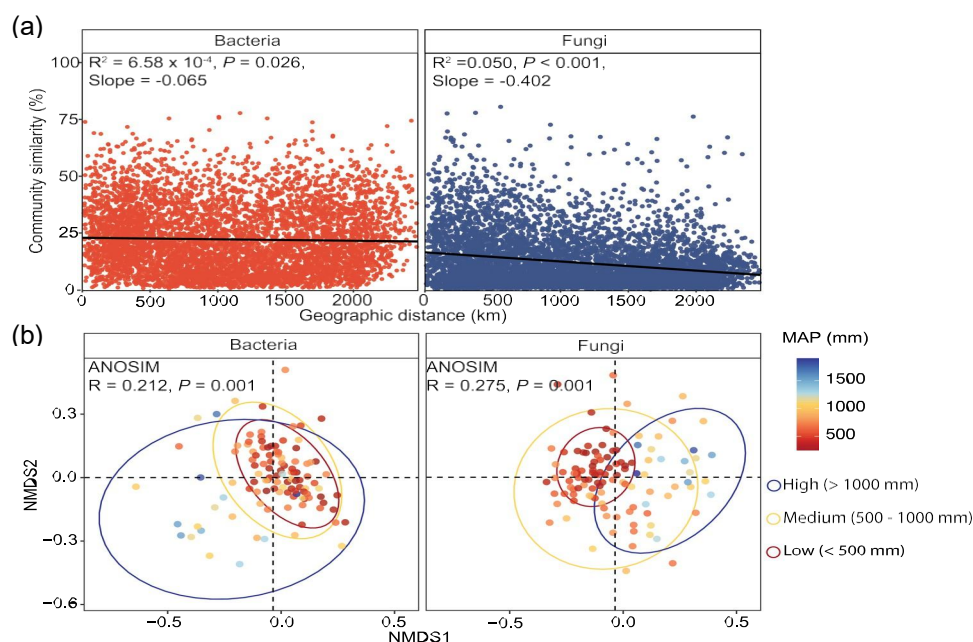


FIGURE 3: To investigate the biogeographic patterns of phyllosphere bacterial and fungal communities, we computed DDRs of the community similarities. The considerable ($p < 0.001$) negative correlations between geographic distance and community similarity (Figure 3a) demonstrated a strong distance decay tendency in both communities. The fungus community's DDR curve was steeper (slope = -0.402) than the bacterial community's (slope = -0.065), suggesting that as geographic distance rises, the fungal population may be more susceptible to environmental changes. NMDS analysis revealed that the community compositions of phyllosphere communities changed considerably (ANOSIM $p = 0.001$) over the gradient of environmental factors, including MAP, MAT, and AI (Figures 3b and S5). Microbial community assembly processes in plant phyllosphere To examine the relative importance of neutral and non-neutral processes in the formation of phyllosphere microbial community assemblies, we fitted bacterial and fungal OTUs to the NCM to estimate the occurrence of a given OTU and its relative abundance. The NCM accounted for 64.2% of the variances in the bacterial community and 48.7% of the differences in the fungal community (Figure 4a). The higher explanation rate of NCM for bacterial communities compared to fungal communities indicates that stochastic processes were more important in the formation of bacterial communities than fungal communities. To better understand the relative significance of stochastic and deterministic events in the phyllosphere microbial community formation processes, we computed NST for bacterial and fungal communities. 50% is used by the NST mathematical framework as the boundary between more stochastic (>50%) and more deterministic (<50%). 40 NST for bacterial and fungal communities was 71.8% and 25.1%, respectively (Figure 4b). These results demonstrated that the bacterial ecology in the phyllosphere was mostly produced by random events.

2.8 | Environmental responses of phyllosphere microbial communities

The findings of Spearman's correlation analysis showed that the fungal community was more affected by environmental conditions than the bacterial community (Table 1). The abundance and community composition (NMDS1 value) of both bacterial and fungal communities were significantly ($p < 0.001$) linked with MAP and AI for climate conditions. MAT was not substantially connected with any feature of the bacterial population, however it was strongly ($p < 0.05$) correlated with fungal abundance and α -diversity. Fungal abundance was substantially ($p < 0.01$) associated with edaphic variables, such as soil pH, dissolved organic carbon (DOC), and dissolved organic nitrogen (DON). Fungal α -diversity was substantially ($p < 0.05$) linked with DON and nitrate concentration. Fungal community compositions were substantially ($p < 0.05$) linked with soil pH, DOC, ammonium, and nitrate concentrations. Nevertheless, the only edaphic component that had a significant ($p < 0.05$) correlation with the bacterial community attribute (abundance) was DON. Results from random forest modelling (ntree = 5000) and variation partitioning analysis (VPA) further showed that fungal community composition was more influenced by environmental variables than bacterial community composition. VPA had an explanation rate of 23% (Figure 5a) and random forest modelling had an explanation rate of 18.35% (Figure 5b), indicating that only a small percentage of the variance in bacterial community composition was explained by the chosen environmental parameters. In contrast, VPA and random forest modelling explained 46% (Figures 5a) and 37.64% (Figure 5b) of the variance in fungal community composition, respectively, indicating that the chosen environmental variables accounted for a substantially greater percentage of the variation in fungal community composition.

3 | DISCUSSION

3.1 | Distinct assembly processes of bacterial and fungal communities

Over the last ten years, our understanding of how microbial communities assemble in natural environments has grown significantly. 2, 6, 44 Nonetheless, factors influencing the phyllosphere of plants

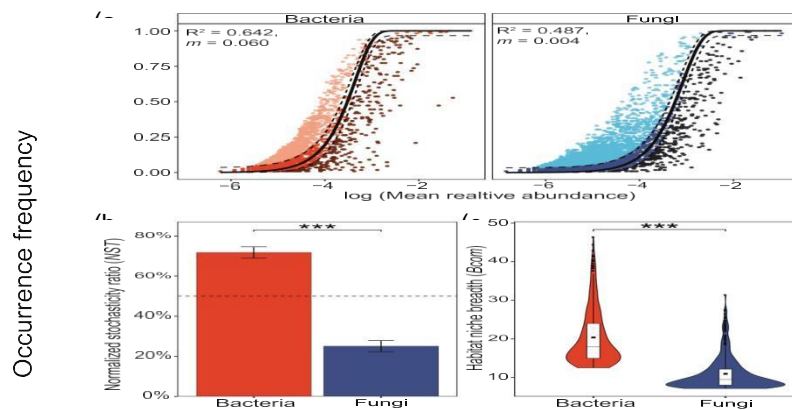


FIGURE 4: (a) The neutral community model (NCM) fit, which displays the expected occurrence frequencies in relation to the relative abundance of fungal and bacterial OTUs. The optimal fit for the NCM is shown by the solid lines. The 95% confidence intervals around the model forecast are shown by the dashed lines. The model's goodness of fit and the predicted migration rate are shown by the R^2 and m values, respectively. (b) Bar graphs that compare the fungal and bacterial populations' normalised stochasticity ratios (NST). (c) The mean habitat niche breadth (Bcom) from all species for each sample in the plant phyllosphere is shown in violin and box plots. Significant differences are indicated by asterisks (*** $p < 0.001$). So far, microbial community assembly have not gotten much attention. This work offered further evidence that the assembly of phyllo-sphere bacterial and fungal communities were dominated by stochastic and deterministic processes, respectively, based on a survey of phyllosphere communities across an almost 4000 km transect. First, we found that the number

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of fungus and bacteria varied by many magnitudes across samples. Furthermore, there was significant variation across samples in the α -diversity (observed species) of phyllosphere microbial communities. These findings suggest that phyllosphere microbial communities are diverse across space since our sample area spans more than 4000 km. Since the phyllosphere is an open habitat, endemic environmental conditions like wind and rainfall may constantly affect phyllosphere communities, which might account for the geographic variety of phyllosphere microbial populations.^{14, 16} Nevertheless, taxonomic classification showed that just two phyla dominated the bacterial and fungal populations in the phyllosphere. This observation is consistent with a prior research that found that since the phyllosphere is a very oligotrophic ecosystem, just a few microbial phyla make up the phyllosphere community, independent of the hostplant species and sample sites.¹⁶ We discovered that the similarity of the composition of the fungal community exhibited a much larger distance decay pattern than the similarity of the composition of the bacterial community (Figure 3a), suggesting that the fungal community may be more affected by environmental filtering than the bacterial community. NMDS ordinations and the noteworthy findings of ANOSIM analysis reinforced the idea that environmental changes had a greater effect on the fungal community than the bacterial population (Figures 3b and S5). The findings showed that a greater percentage of the changes in fungal community composition could be explained by variations in climatic characteristics than by variations in bacterial community makeup. These findings are in line with our earlier research, which shown that stochastic processes contributed more to bacterial communities than to fungal communities since the composition of bacterial communities was less affected by environmental conditions, particularly temperature.⁷ Nevertheless, we discovered in the earlier investigation that deterministic processes predominated both

TABLE 1 Spearman's correlation coefficient between bacterial and fungal community properties and selected environmental factors

	MAP (mm)	MAT (°C)	AI	pH	DOC (mg/k g)	DON (mg/k g)	NH ₄ ⁺ (mg/kg)	NO ₃ ⁻ (mg/k g)
Bacteria								
Abundance (16S rRNA gene copies/g sample)	0.450	0.002	0.385	-0.061	0.231	0.044	0.073	-0.181
	$p < 0.001$	$p = 0.529$	$p = 0.98$	$p < 0.001$	$p = 0.645$	$p = 0.45$	$p = 0.057$	$p = 0.057$
α -diversity (Observed species)	-0.012	-0.054	0.021	-0.072	0.006	-0.015	0.017	-0.067
	$p = 0.902$	$p = 0.578$	$p = 0.831$	$p = 0.456$	$p = 0.953$	$p = 0.873$	$p = 0.862$	$p = 0.485$
Community composition (NMDS1 value)	-0.403	0.035	-0.390	0.084	-0.153	-0.089	-0.098	0.093
	$p < 0.001$	$p = 0.384$	$p = 0.718$	$p < 0.001$	$p = 0.110$	$p = 0.353$	$p = 0.310$	$p = 0.333$
Fungi								
Abundance (ITS copies/g sample)	0.574	-0.232	0.556	-0.267	0.418	0.199	0.034	-0.012
	$p < 0.001$	$p = 0.004$	$p = 0.014$	$p < 0.001$	$p < 0.001$	$p = 0.036$	$p = 0.721$	$p = 0.898$
α -diversity (Observed species)	0.139	0.208	0.096	0.104	-0.055	-0.214	0.019	-0.345

	$p = 0.143$	$p = 0.028$	$p =$	p	$=p$	$=$	p	$=p$	$<$
	0.316	$p = 0.277$		0.562	0.024		0.839	0.001	
Community composition (NMDS1 value)	0.524	-0.121	0.493	-0.198	0.242	-0.062	0.191	-0.378	
	$p < 0.001$	$p = .204$	p	$<$	p	$=p = .515$	p	$=p$	$<$
	0.001	$p = 0.037$			0.010		0.044	0.001	

Note: Significant associations ($p < 0.05$) are shown with bold fonts.

AI stands for aridity index; DOC for dissolved organic carbon; DON for dissolved organic nitrogen; MAP for mean annual precipitation; and MAT for mean annual temperature. assemblies of fungal and bacterial communities in soil environments.⁷ The disparities in the characteristics of the soil and plant phyllosphere may be the cause of the contradicting findings of the ecological processes in determining bacterial community assembly between the two investigations. Furthermore, the degree of community similarity by itself is insufficient to represent the relative significance of stochastic and deterministic processes since both neutral and non-neutral events may affect microbial community similarity.⁴⁵ To further demonstrate the relative significance of stochastic and deterministic processes in forming phyllosphere bacterial and fungal community assemblies, we used NCM to phyllosphere bacterial and fungal communities and computed NST values (Figure 4a,b). The NCM and NST findings repeatedly showed that although niche-based deterministic processes dominated the formation of phyllosphere fungal communities, neutral-based stochastic processes dominated the formation of phyllosphere bacterial communities. The size plasticity hypothesis, which highlights that smaller organisms (bacteria) have greater metabolic plasticity and greater environmental tolerance than larger organisms (fungi), may help to explain the observed differences in the roles of stochastic and deterministic processes in determining the bacterial and fungal community assemblies.^{23, 24} It is well recognised that a small body size is often linked to rapid growth, which increases the likelihood of evolutionary mutation.^{23, 24} Wu et al. (2013) found that the degree of microbial community formation in marine environments controlled by environmental filtration increased with the growing size of microorganisms, which is a significant piece of evidence supporting the size plasticity theory. The size plasticity theory was further confirmed by our findings that bacteria have a much greater habitat niche width than fungus (Figure 4c). Similar findings from our earlier research showed that in termite mound ecosystems, bacteria could adapt to a greater variety of environmental variables than fungus.⁷ These results were consistent, suggesting that the size plasticity concept could be applied to terrestrial and aquatic environments.

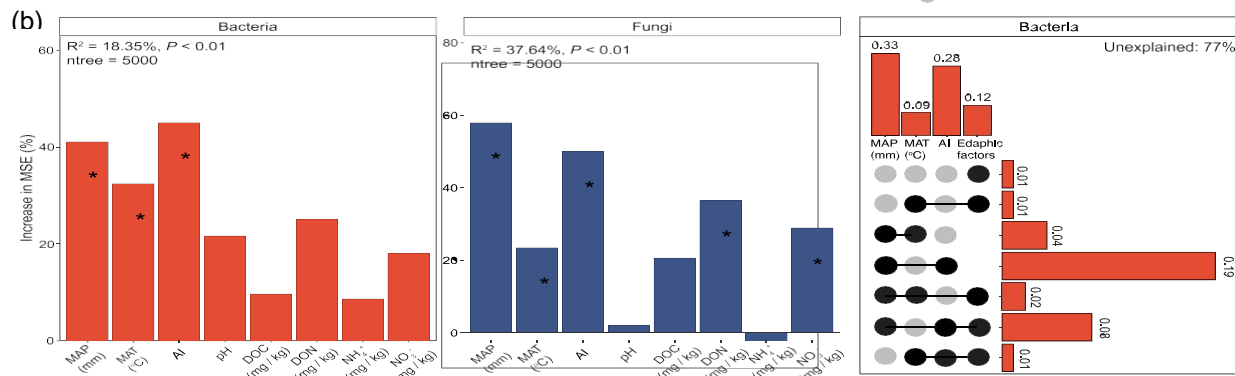
3.2 | Environmental drivers of bacterial and fungal community assemblies

Certain OTUs appeared more or less often than NCM anticipated for both bacterial and fungal communities (Figure 4a). In particular, microbial taxa that were discovered more often than anticipated and could have been actively chosen by environmental filtration are shown by the dots above the neutral prediction line (Figure 4a).⁶ The species that were discovered less often than anticipated and would not be favoured by environmental circumstances are shown by the dots below the neutral prediction line. Six The identification of notable non-neutral community partitions suggests that environmental influences are crucial in shaping the assembly of both fungal and bacterial communities. Microbial populations' reaction to environmental filtration is typically

FIGURE 5(a) Variation partitioning analysis results. The horizontal bar graphs show how much of the change in the microbial community composition (NMDS1 values) may be attributed to the factor or factors shown in the circles on the left panel. The change in microbial community composition (NMDS1 value) explained by a specific factor is shown in the vertical bar graphs at the top. (b) The significance of environmental variables in microbial profile

prediction using Random Forest modelling. MAP stands for mean annual precipitation (mm), MAT for mean annual temperature ($^{\circ}$ C), and AI for aridity index. Significant findings are indicated by asterisks (* $p < 0.05$).

a)



mediated by the equilibrium between deterministic and stochastic processes.^{8, 12, 46}In this work, we found that both bacterial and fungal community compositions were significantly influenced by climatic parameters (i.e., MAP, MAT, and AI), while the effects were distinct for bacteria and fungi. For instance, there was a strong negative correlation between MAP and AI and the makeup of the bacterial community, but a significant positive correlation with the composition of the fungal population (Table 1). The varied stoichiometry, enzymatic capacities, and carbon consumption methods of bacteria and fungus may account for these findings, which were consistent with earlier research in other terrestrial ecosystems such as soil and termite mounds^{7,47,10, 48, 49} Furthermore, we discovered that edaphic variables had little effect on the makeup of bacterial communities but were significant in influencing the composition of fungal communities. Further proof that environmental filtration had a greater impact on the composition of fungal communities than on bacterial communities came from the VPA and random forest modelling findings (Figure 5). Together, these findings suggested that fungus were more vulnerable to the effects of shifting environmental circumstances than bacteria.

4 | CONCLUSIONS

Our combined efforts improved our knowledge of the microbial biogeographic patterns in the phyllosphere. Additionally, this work produced a conceptual paradigm (Figure 6) that illustrates how phyllosphere bacterial and fungal populations assemble and react to environmental filtration. We discovered that stochasticity had a stronger impact on bacterial community formation than on fungal community assembly. This might be because bacteria have a greater capability for migration, a broader range of habitat niches, and more metabolic adaptability than fungi. Furthermore, we discovered that the phyllosphere fungal population was more susceptible than the phyllosphere bacterial community to changes in the soil's DON, ammonium, and nitrate levels. Our findings demonstrate the significance of microbial traits in controlling their ecological assembly processes and reactions to changes in their surroundings. These results may enhance our capacity to forecast how plant microbes and their ecosystem services will react to environmental changes.

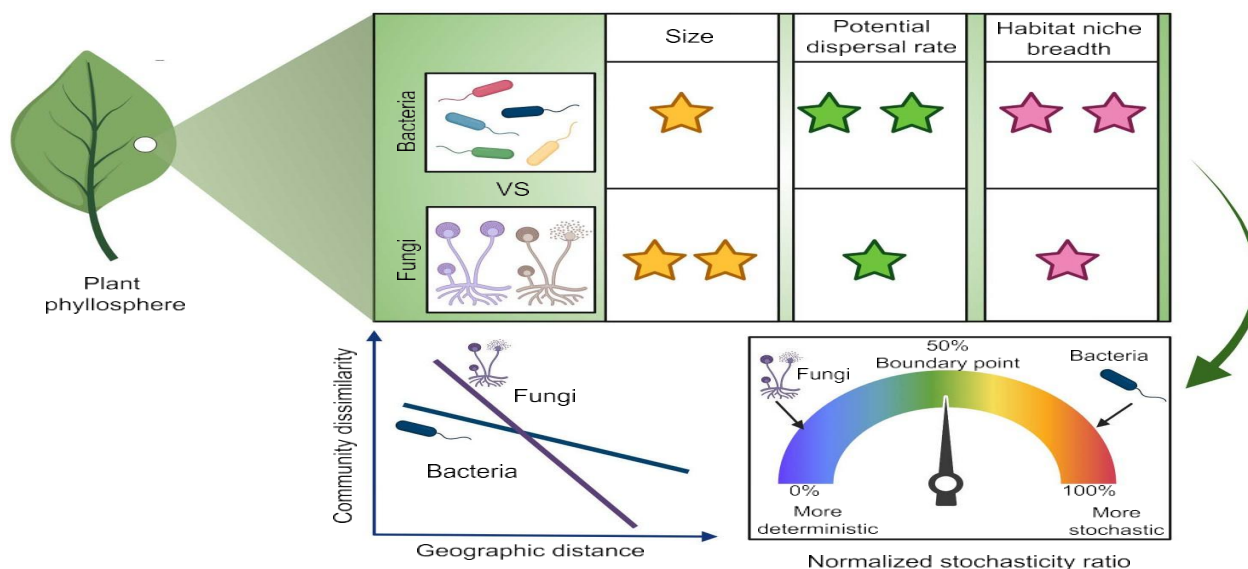


FIGURE 6: The relative significance of deterministic and stochastic factors in determining the bacterial and fungal community assemblages in the plant phyllosphere is summarised schematically. The top graph demonstrates that while bacteria typically have smaller bodies than fungus, they have a greater potential rate of dispersion and a larger range of ecological niches. The bottom left graph shows that phyllosphere bacterial community similarity is less affected by an increase in geographic distance than phyllosphere fungal community similarity. The relative significance of random and deterministic processes in forming phyllosphere bacterial and fungal community assemblies is shown in the bottom right graph.

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